



**CHARACTERIZATION AND EVALUATION OF BANANA PSEUDO-STEMS FOR
BIOETHANOL PRODUCTION**

MSc. THESIS

BY

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HAWASSA UNIVERSITY COLLEGE OF AGRICULTURE

JULY, 2022 G.C

HAWASSA, ETHIOPIA

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**A THESIS SUBMITTED TO HAWASSA UNIVERSITY, SCHOOL OF PLANT
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HAWASSA UNIVERSITY ADVISORS' APPROVAL SHEET

(Submission Sheet – 1)

This is to certify that the thesis entitled **“Characterization and Evaluation of Banana Pseudo-stems for Bioethanol Production”** submitted in partial fulfillment of the requirements for the degree of Masters of Sciences with specialization in Bioenergy Science and Technology, Graduate Program of the School of Plant and Horticultural Sciences, College of Agriculture, Hawassa University and is a record of original research carried out by **Temesgen Ayele Ade**, under my supervision, and no part of the Thesis has been submitted for any other degree or diploma. The assistance and help received during the course of this investigation have been duly acknowledged. Therefore, I recommend that it can be accepted as fulfilling the Thesis requirements.

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First, I declare that this thesis is my own work and that all sources of materials used for the thesis has been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree, the graduate program of Hawassa University, and I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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DEDICATION

This scientific paper is dedicated to my beloved sister Mekides Ayele, my mother Tarikwa Beriso my father Ayele Ade, former head of school of chemical engineering (Hawassa University) Mr. Abiyot Melkamu, and all my family for their incredible support, love and inspiration.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
CV	Coefficient of variation
DC	Dwarf cavendishii
DF	Degree of freedom
ECSA	Ethiopian central statistics agency
EPSE	Ethiopian petroleum supply enterprise
FAO	Food and agricultural organization
FTIR	Fourier transfer infrared
GC	Giant cavendishii
GDP	Growth and transformation plan
MS	Mean square
NREL	National renewable energy laboratory
SAS	Statistical analysis software
SGS	School of graduate studies
SNNPR	South nation's nationalities and peoples region
SS	Sum square
TAPPI	Technical association of pulp and paper industry
UN	United Nations
W1	William-1

ABSTRACT

Bio-fuels like bioethanol originated from lignocellulosic biomasses are being investigated as potential substitutes for current high pollutant fuels obtained from conventional sources. Banana pseudo-stem is one of lignocellulosic biomass, which is generated from banana cultivation. This study was carried out to Characterize and Evaluate Banana Pseudo-stems for Bioethanol Production. For this study, Giant Cavendishii (M. acuminata), Dwarf Cavendishii (M. acuminata), and William-1 (M. 'Williams hybrid') banana pseudo-stems were used for investigation. In this study, chemical compositions of pseudo-stems (extractives, cellulose, hemicellulose, lignin, and ash) were determined through National Renewable Energy Laboratory (NREL) protocol. Bioethanol was also produced from each pseudo-stem through biochemical conversion method. The production method had four major processes; pseudo-stem pretreatment, hydrolysis, fermentation, and distillation. Some of the physicochemical properties of produced bioethanol (Viscosity, Density, Flash point, Alcohol concentration, and Calorific value) were tested. The functional group of the produced bioethanol was analyzed using Fourier Transform Infrared (FTIR) spectroscopy. And finally, bioethanol yield of each variety was determined depending on the concentration of produced bioethanol. Each laboratory experiments were conducted for triplicate. The data analysis of experimental result was done by using statistical analysis of variance (one way ANOVA), through statistical analysis software (SAS). The laboratory result of extractives, cellulose, hemicellulose, lignin, and ash content of the pseudo-stems were 27.25% - 31.15%, 30.11% - 36.14%, 19.32% - 23.83%, 8.81% - 9.30%, and 7.99% - 9.30%, respectively. And bioethanol yield of Giant Cavendishii, Dwarf Cavendishii, and William-1 pseudo-stem were 6.31%, 5.20%, and 4.00%, respectively. The statistical analysis software (SAS) output implied that the pseudo-stems of the three varieties have significantly different cellulose composition and bioethanol yield. As the result implied, pseudo-stem of giant cavendishii (M. acuminata) has the largest cellulose content and bioethanol yield followed by dwarf cavendishii (M. acuminata). Thus, giant cavendishii (M. acuminata) has relatively higher bioethanol potential. Therefore, giant cavendishii banana pseudo-stem is recommended to use as feedstock for bioethanol production.

Key words: *Banana Pseudo-stem, Bioethanol, Bioethanol yield, Biofuel, dwarf cavendishii, giant cavendishii, Lignocellulosic biomasses, William-1*

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The growing demand for energy, rising world fuel prices, and concerns about global warming are the key factors driving the increasing interest in renewable energy (Mehling, 2017). Renewable energy is energy which is extracted (derived) from renewable natural resources; can be renewed or replenished in a reasonable amount of time (in years or a human-life span), once it has been used (Randall, 2018). It is generated from natural sources like sun, wind, rain, tides, and biomasses (Georgia, 2014).

Biomasses are organic matters of recent origin produced directly or indirectly by living organisms. Within biomass, the light energy of sunlight is store in the form of chemical energy. In plant matter, biomass is produced by the process of photosynthesis in which carbon dioxide and water from the environment are converted, using energy from sunlight. It is converted into carbohydrates (sugars, starches, cellulose, hemicellulose, and lignin) that constitute the plant (Ness & Moghtaderi, 2016). Also, organic waste produced by animals which feed on the plants and/or other animals are a form of biomass. So that, biomass may then be regarded as an energy resource which can be used to provide heat, electricity, and transport fuels, collectively they are called Bioenergy (Carrillo-Nieves *et al.*, 2019).

The name 'Bioenergy' is the combination of the terms biomass and energy. It is a form of renewable energy generated (derived) from the conversion of biomass into heat, electricity, biogas and liquid fuels. Bioenergy is also the term commonly used to refer the chemical

energy stored in biofuels. Biofuels are solid, liquid or gaseous fuels made from the wide range of organic materials identified as biomass. Some biofuels such as wood and fibrous materials may be used directly as fuel sources with little processing, whereas other biofuels are obtained indirectly from the biomass through an appropriate conversion technology to provide the energy in a form more convenient to deliver the required energy service (Ness & Moghtaderi, 2016).

Bioethanol and biodiesel are the most common types of liquid biofuels used for transportation. Bioethanol is the most popular liquid biofuel which is produced from any feedstock containing significant amounts of sugar, through fermentation (Benti *et al.*, 2021). It is an attractive alternative fuel to substitute gasoline or uses by blending with gasoline. Bioethanol is believed to have a promising future in tackling today's global energy crisis and worsening environmental protection. Bioethanol from corn and sugarcane dominate the current biofuels market (Deshavath *et al.*, 2019). But due to its impact on food price and food security, starch and sugar-based feedstocks cannot be recommended for bioethanol production. To mitigate this circumstance, the world has been shifting to develop second-generation biofuels made from non-food feedstocks.

The production and use of bioethanol has been increasing significantly in many countries around the world. In recent year, the United States of America and Brazil become the leading contributors of global bioethanol production. In the year 2020, United States of America produce over 13.9 billion gallons and Brazil produce around 9 billion gallons of bioethanol (Renewable fuels association, 2021). Together, the USA and Brazil produce 84% of the world's bioethanol. Bioethanol production in Ethiopia is linked with sugar factories and aimed

for import substitute of petroleum products (Benti *et al.*, 2021). It is produced from molasses, the byproduct of sugar. Finchaa and Metehara sugar factories are the only sugar factories producing and supply bioethanol in Ethiopia (Benti *et al.*, 2021). In 2015 about 20.5 million liters of bioethanol was supplied to the energy system of the country and all used in the transport sector (Gabisa & Gheewala S.H., 2018). In the near future, the existing (such as wonji/shoa) and new sugar factories under construction together are expected to start producing bioethanol. The total annual production must be expected to rise up to 182 million liters when the factories start bioethanol production (Gebreyohannes, 2013). Similar with increasing bioethanol production, the demand is expected to increase rapidly. Because of the ongoing rise on ethanol demand and overconsumption of fossil fuels, exploitation of lignocellulosic biomasses like agricultural waste is mandatory to cover the upcoming demand.

In Ethiopia, there are many different types of agricultural wastes; banana pseudo-stem is one of an agricultural wastes generated from banana cultivation. Banana is an important fruit crop all over the world, providing sufficient carbohydrates with high nutrition, and the plant produce enormous pseudo-stem residues which is rich in lignocellulose (Guerrero *et al.*, 2015). The plant is composed of the corm (rhizome or bulb), pseudo-stem, leaves, bunch and root. The pseudo-stem is the cylindrical part of the plant, which makes about 75% of the total waste generated from banana cultivation (Anhwange *et al.*, 2009). Banana pseudo-stem consisted of considerable amount of cellulose and hemicellulose and lower lignin (Islam *et al.*, 2019).

Moreover, after harvesting banana fruits, the pseudo-stem is cut and left as waste. Ethiopian central statistics agency 2020 report showed that, from the production of 501,528.63 tons of

banana fruit, 1.505 million tons of banana pseudo-stems were generated. The highest lignocellulosic content and its abundance make the pseudo-stem to be preferred feedstock for bioethanol production. Because of this, the present study was conducted to determine the banana pseudo-stem with higher bioethanol potential by taking the pseudo-stems of three most popular and commonly cultivating banana varieties in Ethiopia. Those varieties are giant Cavendishii (*M. acuminata*), dwarf Cavendishii (*M. acuminata*), and William-1 (*M. 'Williams hybrid'*).

1.2 Statement of the Problem

Energy is a key factor which determines the sustainable development of the countries and it is the back-bone of the economy. Different studies imply that the need of energy in farms, industries and transport sectors is substantially increasing through time. To satisfy the increasing energy demand, fossil fuel has been extensively used as source of energy even though burning of fossil fuel is the major source for greenhouse gas emission causes global warming.

In Ethiopia, the largest share, up to 92.2% share of the total primary energy demand is fulfilled from biomass combustion where household demand is the majority (Tiruye *et al.*, 2021). Our excessive dependency on only woody biomass as energy sources leads us to the loss of huge forest coverage. The U.N. FAO 2011 report shows that, in total, between 1990 and 2010 Ethiopia lost 18.6% of its forest cover, which is around 2,818,000 hectares. On the other hand, the transportation sector is also provided by importing the fossil fuel. Thus, Ethiopia has been investing over 80% of its foreign earnings annually to import the entire petroleum fuel requirement (Benti *et al.*, 2021). According to Ethiopian petroleum supply enterprise (EPSE)

2020 report, Ethiopia has imported close to 81.8 billion-birr (around \$2.82 billion) fuel in the year 2019.

Bioethanol is one of biofuels which is used as transportation fuel instead of gasoline or by blending with it. It is also used as cooking fuel by blending with kerosene. Bioethanol is produced from varieties of biomasses; lignocellulosic materials are the most abundant and cheapest one. Banana pseudo-stem is one of lignocellulosic biomass which is an agricultural waste after harvesting banana fruit. In Ethiopia, every year after harvesting banana fruits, a large amount of bare pseudo-stem is cut and left behind as waste. According to Ethiopian central statistics agency 2020 report, the 2019 total annual banana fruit production of Ethiopia was 501,528.629 tons. According to Abbi *et al.*, (1996) study, from the production of this amount of banana fruit, 2.006 million tons of agricultural residues (75% or 1.506 million ton pseudo-stem) was generated. Standing on Guerrero *et al.*, (2015) study, it is possible to produce around 203 million liters of bioethanol from this biomass. Therefore, production of bioethanol from banana pseudo-stems to use as transportation and cooking fuel is one of the ways to reduce both environmental pollution due to deforestation and crude oil consumption. Thus, this study was initiated to determine the bioethanol potential on the pseudo-stems of three banana varieties (most commonly cultivated varieties in SNNPR, Gamo zone).

1.3 Objectives

1.3.1 General objective

The general objective of this study was to characterize and evaluate banana pseudo-stems for bioethanol production.

1.3.2 Specific objectives

The specific objectives of this study were:

- ❖ To determine chemical compositions of Giant Cavendishii (*M. acuminata*), Dwarf Cavendishii (*M. acuminata*), and William-1 (*M. 'Williams hybrid'*) banana pseudo-stems.
- ❖ To produce bioethanol from Giant Cavendishii (*M. acuminata*), Dwarf Cavendishii (*M. acuminata*), and William-1 (*M. 'Williams hybrid'*) banana pseudo-stems.
- ❖ To characterize the bioethanol produced from the three varieties of banana pseudo-stems.
- ❖ To compare the bioethanol yield obtained from banana pseudo-stems of the three varieties.

1.4 Significance of the study

The study will help to identify the best variety of banana pseudo-stem with highest bioethanol potential. The investigation of this study will also contribute its own role for Ethiopian bioenergy sector researchers, via being a source of information on composition of the three pseudo-stem varieties. This study would serve as a foundation for related researchers who are working on banana pseudo-stem to produce bioethanol.

1.5 Scope of the study

This study was carried out to identify the best banana pseudo-stem variety with higher bioethanol potential. The study was carried out through four major activities. First, chemical compositions of the pseudo-stems (extractives, hemicellulose, cellulose, lignin, and ash) were analyzed according to national renewable energy laboratory (NREL) protocol. Secondly, bioethanol from each variety was produced through separate saccharification and fermentation

using yeast (*S.cerevisiae*). At the third, some of the physico-chemical properties of produced bioethanol (density, viscosity, flash point, alcohol concentration, and calorific value) and alcohol or O-H identification on the produced bioethanol was done via FT-IR spectroscopy, and finally, the bioethanol yield was calculated and the result was compared with each other by using SAS software through one way ANOVA. For the present study, the pseudo-stems used were collected from Arba Minch zuria wereda, Chano district and dried samples were used for all analysis.

CHAPTER TWO

LITERATURE REVIEW

2.1. Energy

Energy is a key topic today in science and technology. Regarding energy, a frequently found definition in common literature as well as in textbooks is “energy is the ability to do work” or slightly more detailed “energy is the capacity of a system to do work” (Mehling, 2017). Since starting from ancient time, humans use energy by converting between the different energy forms for the need of their daily life as well as for industrial applications. The world today cannot walk a single step without energy. Because of this, more sophisticated and modernized technologies are emerging to exploit energy stored in every resource (Deshavath *et al.*, 2019). Based on their sources from which they recovered (derived), energy must be classified as renewable and non-renewable energy.

Renewable energy is energy that is derived from renewable resources that are naturally replenished on a human timescale; includes sources such as sunlight, wind, rain, tides, waves, geothermal heat and biomass (Ellabban *et al.*, 2014). Renewable energy often provides energy in four important areas: electricity generation, air and water heating/cooling, transportation, and rural (off-grid) energy services (Ellabban *et al.*, 2014). Despite of renewable, non-renewable energy is obtained from non-renewable natural resources. The resources have specific conditions that made them, and can take many generations to reproduce. Sometimes the conditions are not likely to occur again so they are limited in supply and once used cannot be re-generated within a short span of time (Georgia, 2014). The primary sources for non-renewable energies in the world are fossil fuels: coal, natural gas, and oil (Randall, 2018).

2.2. Bioenergy

Bioenergy is renewable energy that is derived from recently living organic materials, such as wood, agricultural crops or organic waste. It is obtained from recently grown organic material, known as biomass, which can be used to produce transportation fuels, heat, and electricity. Overall, bioenergy covers approximately 12% of the total world energy demand (World Bioenergy Association, 2021). Traditional unprocessed biomass such as fuel-wood, charcoal and animal dung accounts for most of this and represents the main source of energy for a large number of people in developing countries who use it mainly for cooking and heating. Sometimes 'Bioenergy' is defined as energy derived from biofuels.

Biofuels are fuels produced directly or indirectly from organic material including plant materials and animal waste. They are energy carriers that store the energy derived from organic materials (called biomass). Biofuels may be solid, such as fuel-wood, charcoal and wood pellets; liquid, such as ethanol, biodiesel and pyrolysis oils; or gaseous, such as biogas (Deshavath *et al.*, 2019). Often the term biofuel is used in a narrow sense to refer to liquid biofuels for transport (Benti *et al.*, 2021).

2.3. Bioethanol

Bioethanol and biodiesel are the two most commonly used biofuel types (Benti *et al.*, 2021). Bioethanol (ethyl alcohol, grain alcohol, $\text{CH}_3\text{-CH}_2\text{-OH}$, or ETOH) is a liquid biofuel that can be produced from various biomass feedstocks and conversion technologies (Benti *et al.*, 2021). It is a type of alcohol that can be produced from any feedstock containing significant amounts of sugar (such as sugar cane or sugar beet), starch, (such as maize and wheat), or cellulosic (such as sugar cane bagasse, rice hulls) through fermentation. Sugar can be directly fermented to alcohol, starch needs to break down (hydrolyze) it in to sugar before

fermentation, and lignocellulosic materials need pretreatment in addition to hydrolysis. The fermentation process is similar to that used to make wine or beer, and pure ethanol is obtained (recovered) by distillation.

Because of its higher octane number, broader flammability limits, higher flame speeds and higher heats of vaporization than gasoline (Bailey, 2018), bioethanol is becoming an attractive alternative transportation fuel. This provides the potential to reduce particulate emissions in spark-ignition engines (Benti *et al.*, 2021). Bioethanol can be blended with petrol or burned in nearly pure form in slightly modified spark-ignition engines. A liter of ethanol contains approximately two thirds of the energy provided by a liter of petrol. However, when mixed with petrol, it improves the combustion performance and lowers the emissions of carbon monoxide and sulphur oxide (Carrillo-Nieves *et al.*, 2019).

Bioethanol is deemed as one of the excellent energy resources that attribute for the spark-ignition internal combustion engines. It contains higher octane and higher heat associated with vaporization. It has a high compression ratio, reduced burning time and lean burn engine, which has made it superior over gasoline (Splitter *et al.*, 2016) and (Carrillo-Nieves *et al.*, 2019). Octane number measures the engine performance, wherein, a higher number indicates better combustion.

As reviewed by Benti *et al.*, (2021), after combustion, ethanol emits less quantity of particulate matters, hydrocarbons and NO_x because of the oxygen content of ethanol i.e. 35% oxygen. Moreover, the octane number and combustion efficiency of bioethanol are higher than gasoline. Properties of ethanol are given in Table 1. As per Toor *et al.*,(2019), “Despite advantages, bioethanol has some shortcomings such as lesser energy density, small flame

luminosity, corrosive nature, lesser vapor pressure (making cold starts difficult), water miscibility and toxicity to ecosystems”.

Table 1: Typical physicochemical properties of bioethanol (99.5%) (Toor et al., 2019)

Properties	Unit	Value
Density	g/cm ³	0.789
Cloud point	°C	>8
Flash point	°C	13.5
Viscosity	cSt@25°C	1.2
Sulphur	Ppm	-
Cetane number		5-8
Avg. Molecular weight	kg/mol	46
Boiling Point	°C	78
HHV(CV)	MJ/kg	29.2

2.3.1. Bioethanol status in Ethiopia

Like many African countries, Ethiopia heavily relied on biomass-based energy by consumers for household purposes and imported fossil fuels for transportation sector. The largest energy supply comes from waste and biomass covering up to 92.2% share of the total primary energy supply followed by oil (5.7%) and hydropower (1.6%) (Tiruye *et al.*, 2021). Biofuels are regarded as an opportunity to ensure domestic energy security, rapid economic growth and wealth creation (Benti *et al.*, 2021). There are high expectations that biofuels will contribute to

solving the country's main development challenges today (Gebreegziabher *et al.*, 2014). In Ethiopia, almost all the feedstocks needed for the production of bioethanol (sugarcane, sugarbeet, cereals, and maize) are grown.

Bioethanol production in Ethiopia is linked with sugar factories and aimed for import substitute of petroleum products (Benti *et al.*, 2021). It is produced from molasses, the byproduct of sugar. The production is less expensive since the fermentation process could be made straight away. According to Mengistu, (2013) study, from 1 ton of crushed cane, about 3–4% final molasses can be found; and 1 ton of molasses can produce about 250 L of bioethanol. Finchaa and Metehara sugar factories are the only two sugar factories producing bioethanol in the country (Benti *et al.*, 2021). In 2015 about 20.5 million liters of ethanol was supplied to the energy system of the country (8 million liters from Fincha sugar factory, whereas about 12.5 million liters per year were from the Metahara sugar factory) using molasses as feedstock and all used in the transport sector (Gabisa & Gheewala S.H., 2018).

However, only a small fraction of the potentials are utilized yet and an alternate 5% and 10% ethanol blend has been accessed in the capital city of the country. The Ethiopian sugar corporation has planned to intensify the annual production of bioethanol to 28.1 million liters by the end of the second GDP from the existing factories and others like; Tandaho and Wonji/Shewa expansion projects, and from recent sugar factories (Ethiopia, 2021). Mostly, bioethanol produced from sugar factories has been used in the transport sector by blending with gasoline. Currently, there are three bioethanol blending stations in Ethiopia, Nile Petroleum, Oil Libya, and National Oil Company (Mengistu, 2013).

2.3.2. Feed-stocks for bioethanol

Bioethanol feedstocks can be conveniently classified into three types:

- (i) Sucrose-containing feedstock
- (ii) Starchy materials and
- (iii) Lignocellulosic biomass

Sucrose-containing feedstocks: Sugar cane, sugar beet, and sweet sorghum are the most common sucrose containing feedstock used for bioethanol (Balat *et al.*, 2008). The conversion of sucrose into bioethanol is easier compared to starchy materials and lignocellulosic biomass; because previous hydrolysis of the feedstock is not required since this disaccharide can be broken down by the yeast cells. In addition, the conditioning of the cane juice or molasses favors the hydrolysis of sucrose (Dien *et al.*, 2003).

Starchy materials: Starch is defined as a polymer consisting only one monomer, D-glucose (Pongsawatmanit *et al.*, 2007). It consists of long chains of glucose molecules and can also be converted to fermentable sugar by a method called “hydrolysis”. To produce bioethanol from starch (e.g. wheat, corn, and barley), it is necessary to break down the chains of this carbohydrate to obtaining glucose syrup, which can be converted into bioethanol via fermentation using microorganisms like yeasts.

Lignocellulosic biomass: Lignocellulosic biomass, such as agricultural residues (corn stalk and wheat straw), wood and energy crops, is an attractive material for bioethanol production since it is the most abundantly reproducible resource on the Earth (Balat *et al.*, 2008). The basic structure of all lignocellulosic biomass consists of three basic polymers: cellulose ($C_6H_{10}O_5$)_x, hemicelluloses such as xylan ($C_5H_8O_4$)_m, and lignin [$C_9H_{10}O_3-(OCH_3)_{0.9-1.7}$]_n (Arin & Demirbas, 2004) and (Demirbas, 2005).

2.4. Bioethanol production from lignocellulosic biomass

For the term Bioethanol, “Bio” refers the source of Ethanol; it is from biological feedstocks called Biomass. Ethanol is also known as ethyl alcohol with the chemical formula C_2H_5OH , and it is a flammable, clear, colorless and slightly toxic chemical compound with acceptable odor. It can be produced either from petrochemical feedstock by the acid-catalyzed hydration of ethene or from biomass which are reach in carbohydrate through fermentation.

Bioethanol production from lignocellulosic biomass encompasses four major processes:

1. Feedstock pretreatment
2. Hydrolysis
3. Fermentation and
4. Distillation

2.4.1. Feedstock pretreatment

Pre-treatment is required to break down the crystalline structure of the cellulose and separating the cellulose away from the lignin in the cell walls, to facilitate for hydrolysis. The goal of pretreatment is to make the cellulose accessible to hydrolysis for conversion to fuels. In practice, several types of pretreatment processes may be necessary to reduce raw biomass stock to a state where fermentable sugars can be extracted and converted to ethanol. The most appropriate pretreatment process could vary with the feedstock since different feedstocks will differ in their content of the different components (cellulose, hemicelluloses and lignin) (Swiatek et al., 2020).

Pretreatment processes of lignocellulose are necessary to break down lignin and increase accessibility of enzymes and microbes to carbohydrates (Demirbas, 2005) and (Hendriks &

Zeeman, 2009). There are a variety of pretreatment techniques including; physical pretreatments, physico-chemical treatments, chemical (acid and alkaline) and biological pretreatment (using fungi and actinomycetes) (Taherzadeh & Karimi, 2008). During acid-catalyzed pretreatment, only low amounts of lignin are removed in forms of soluble fragments (Swiatek *et al.*, 2020). As Kusmiyati & Sukmaningtyas, (2018) study, for banana pseudo-stem, alkaline pretreatments are more effective to dissolve lignin than acid, and also decrease cellulose crystallinity and increasing access for enzymes.

2.4.2. Hydrolysis

Hydrolysis is a process it involves breakdown of the polysaccharides to their simple sugar. The cellulose molecules are composed of long chains of glucose molecules. In the hydrolysis process, these chains are broken down to "free" the sugar, before it is fermented for alcohol production. There are two major type of hydrolysis processes: a chemical reaction (acid hydrolysis), and an enzymatic reaction (Balat *et al.*, 2008).

I. Acid hydrolysis

Proper combinations of pH, temperature, and reaction time can result in high yields of sugars (Carlos *et al.*, 2013). There are two types of acid hydrolysis process commonly used - dilute and concentrated acid hydrolysis.

A. Dilute acid hydrolysis

The dilute acid process is conducted under high temperature and has reaction time in the range of seconds or minutes (Karim *et al.*, 2014). Hydrolysis with dilute sulfuric acid at moderate temperatures has proven to be an efficient means of producing glucose from cellulose ($\text{H}_2\text{SO}_4 < 1\%$, 215 °C and 3 min) (Mushimiyimana & Tallapragada, 2016).

B. Concentrated acid hydrolysis

The concentrated acid process uses relatively mild temperatures, but at high concentration of sulfuric acid (Karim *et al.*, 2014). Despite of dilute acid, in concentrated acid hydrolysis, reaction times are typically much longer (H_2SO_4 , 30–70%, 40°C, a few hours to achieve >90% glucose yields) (Mushimiyimana & Tallapragada, 2016).

I. Enzymatic hydrolysis

Enzymatic hydrolysis of pretreated lignocellulosic materials involves enzymatic reactions that convert cellulose into glucose and hemicellulose into pentose (xylose and arabinose) and hexoses (glucose, galactose, and mannose)(Carlos *et al.*, 2013). The conversion of cellulose and hemicellulose is catalyzed by cellulase and hemicellulase enzymes, respectively. The enzymes are highly specific. The enzymatic hydrolysis is usually carried out at mild conditions (pH 4.8 and temperature 45– 50°C). Enzymatic hydrolysis of cellulose is affected by factors like substrates, cellulase activity, and reaction conditions such as temperature, pH, and other parameters (Araguirang *et al.*, 2020).

2.4.3. Fermentation

Fermentation is the mechanism where a number of microorganisms transform carbohydrates, such as glucose, into ethanol and carbon dioxide (Benti *et al.*, 2021). This is the chemical transformation of organic substance into simpler compounds by the action of microorganisms (yeasts, bacteria and fungi can ferment sugars to produce ethanol.). Originally the term fermentation was used to mean the enzymatic breakdown of carbohydrates in the absence of air. In industrial practice, fermentation refers to any process by which raw materials are transformed by the controlled action of carefully selected strains of organisms into definite

products (Balat *et al.*, 2008). Louis Pasteur used the term in a narrower sense to describe changes brought about by micro-organisms growing in the absence of air. However, for the cause of this thesis it is a biological method of producing ethanol. The fermentation reaction is caused by yeast or bacteria which needs simple sugars. The glucose produced from the hydrolysis is fermented with yeast to produce ethanol and carbon dioxide as chemical reaction shown below.



2.4.4. Distillation

Distillation is a unit operation, or a physical separation process, not a chemical reaction. It is broadly defined as the separation of more volatile components from less volatile components by a process of vaporization and condensation. The term distillation is properly applied only to those operations where vaporization of a liquid mixture yields a vapor phase containing more than one constituent and desired to recover one or more of these constituents in a nearly pure state. The process of distillation is affected based on the relative volatilities of the liquids in the mixture and taking advantage of their different boiling point (Balat *et al.*, 2008). Distillation is the most widely used method of separating liquid mixture and is at the heart of separation process in many chemical and petroleum plants. Bioethanol separation through distillation is carried out at a temperature of 78°C and a distillation time of 50 to 60 minutes by distillation units (Hossain *et al.*, 2019). Bioethanol is recovered in the distillation unit and also concentrated up to 99.99%.

2.5. Banana plants

Banana is one of the most important fruit crops in the world. It is the fourth most important crop of the food market next to rice, wheat, and maize (Gebre *et al.*, 2020). It is the largest of

the herbaceous plants, its pseudo-stem reaching a height of 2.21 to 7.6m in cultivated varieties and the impressive aerial shoot is borne on the subterranean stem which is about 30 cm long (Nelson *et al.*, 2006) and (Ambisa *et al.*, 2019). Banana also have the largest leaf area, which varies between cultivars and depends upon growing conditions (Eldad *et al.*, 2016). The plant bears one bunch in its life, leaving behind a large amount of agricultural residues (starchy and lignocellulosic biomass), which could be used for different purposes such as biofuel production (Guerrero *et al.*, 2015).

Originally, there were two wild species of banana plants, *M. acuminata* and *M. balbisiana*. With time, these two species hybridized to generate the numerous cultivars available today (Mbwana *et al.*, 1998). The genetic *M. acuminata* types of bananas (AA) originated in Malaysia and hybridization between sub-species gave rise to a range of diploid (AA) and triploid (AAA) cultivars. The *M. balbisiana* types (BB) originated in the Indian sub-continent. The spread of AA-types through Southeast Asia to the Indian sub-continent led to hybridization between AA and BB types which gave rise to the AAB and ABB cultivars that are grown worldwide today. Bananas with the AA or AAA genomes are typically sweet and cultivated as dessert banana. Hybrids with AAB and ABB genomes are starchier and therefore used as cooking bananas. AAB and ABB hybrids are grown in coastal lowlands, while the AAA-type well grows at elevations of 1000-1800 meters above sea level. The AAA-varieties are widely grown in the East African highlands; it includes Ethiopia (Mbwana *et al.*, 1998). Overall, there are believed to be approximately 1000 varieties of cultivated bananas (Net, 2016). They are derived from two species of the genus *Musa*, namely *M. acuminata* and *M. balbisiana*.

In Ethiopia, even though both dessert and cooking types of banana are released by the research system; the types that are under production are dessert type that has been under production since the early 1970s. In major banana producing areas, farmers produce formerly recommended varieties such as Dwarf Cavendishii, Giant Cavendishii, William-1 and Poyo (Ambisa *et al.*, 2019). Dwarf cavendishii and Giant cavendishii are most popular varieties produced in large amount for market. Physiologically, those varieties are different; Dwarf cavendishii has short plant height (up to 2.21m), Giant cavendishii is relatively taller (3.4m and more) than Dwarf and William, and also William-1 has medium height (2.90m) (Ambisa *et al.*, 2019).

Banana is mainly produced in the southern and southwest part of the Ethiopia; in South Nations, Nationalities and Peoples Regional State (SNNPR) and Oromiya Regional State. Specifically, Gamo zone is the major banana production area in the country (Ambisa *et al.*, 2019). Southwest part of the country around Jimma, Sheka, Kaffa, and Bench Maji zones in Southern and Oromiya regional state are also banana producing areas. In Amhara, Tigray, Benishangul- Gumuz, and Gambella regions banana is cultivated as a garden crop, mainly for domestic consumption (Ambisa *et al.*, 2019). To differentiate potential areas and banana production trend, the regional production level of 2004, 2005, and 2006 are shown below in table 2. The recent information's are also show the rapid increment of banana cultivation. In the year 2017/2018 annual banana production of Ethiopia was 493,602.234 tons (CSA), 2018). The production has been increasing year up to year. The 2020 Ethiopian central statistics agency report showed that the 2019 total annual banana production was increased to 501,528.629 tons.

Table 2: Regional production level of banana (quintals)(Source: Ethiopian central statistics agency 2006 report)

Region	2004	2005	2006
Tigray	-	-	74.23
Afar	-	-	42,315.47
Amhara	704	674	18,912.96
Oromiya	451,683	451,683	642,393.3
Somali	6545	5909	-
Benishagul Gumez	18618	27537	15426.78
SNNP	549,148	589,833	1,353,977
Harari	618	179	-
Total	1,032,396	1,075,815	2,073,100

2.6. Banana pseudo-stem

A banana plant is composed of the corm (rhizome or bulb), pseudo-stem, leaves, bunch and root. The plant is normally tall and fairly sturdy; as a result it is often mistaken for a tree. However, the trunk of the banana plant is actually a false stem or pseudo-stem. The pseudo-stem is the stem of banana plant (cylindrical part); it produces a single bunch of bananas before die and then is replaced by new pseudo-stem (Anhwange *et al.*, 2009). Banana pseudo-stem is normally 2.21 to 7.6 meters tall (varies from species to species) growing from a corm (Ambisa *et al.*, 2019) and (Nelson *et al.*, 2006). The pseudo-stem consists of a tender core and several outer sheaths. The tender core inside the pseudo-stem carries the immature inflorescence until eventually it emerges at the top. Since each plant produces only one bunch

of bananas and cannot be used for the next harvest, this agricultural activity generates a large amount of residue (Cordeiro *et al.*, 2004). Therefore, every year after harvesting, a large amount of bare pseudo-stem is cut and left behind as waste worldwide (Aziz *et al.*, 2011). As Abbi *et al.*, (1996) study, for the production of 1 ton of banana fruit 4 tons of agricultural residues are generated from which 75% is the pseudo-stem. Depending on this study, in the year 2019, 1.506 million tons of pseudo-stems were generated in Ethiopia.

2.6.1. Composition of banana pseudo-stem

As every lignocellulosic biomass, banana pseudo-stem is composed of a matrix formed of several organic polymers; extractives, hemicellulose, cellulose, lignin, and ash (minerals).

Extractives: Extractives in biomass refer to the non-structural components such as fats, waxes, proteins, terpenes, gums, resins, simple sugars, starches, phenolics, essential oils, pectins, mucilages, glycosides and saponins, fatty acids, sterols, and flavonoid that can be extracted by solvents (e.g. water, ethanol, acetone, benzene, hexane, dichloromethane, and toluene). Extractives constitute 9.7 to 24.1% in dried banana pseudo-stems (Abdullah *et al.*, 2014) and (Guerrero *et al.*, 2015).

Hemicellulose: Hemicellulose is the second most abundant polymer of plant cell walls and is mainly composed of xylan and mannan (Rocha-Meneses *et al.*, 2017). It is a mixture of various polymerized monosaccharides such as glucose, mannose, galactose, xylose, arabinose, 4-O-methyl glucuronic acid and galacturonic acid residues. Hemicellulose accounts 8.7% to 36.0% of dried banana pseudo-stem see in table 3. Compared with cellulose, hemicellulose has a lower degree of polymerization. It is chemically heterogeneous, and also has a random and amorphous structure (Yu *et al.*, 2007). As hemicellulose is wrapped around

the cellulose fibrils, it needs to be removed in order to increase the cellulose digestibility. Thus, increase in hemicellulose removal increases the accessibility of the cellulose and its hydrolysis rate (Zabed *et al.*, 2016).

Cellulose: is the most abundant organic component on earth (Rocha-Meneses *et al.*, 2017). It has a flat ribbon like structure made up of thousands of glucose molecules, which are linked together. Cellulose is a polymer of 6-carbon sugar molecule (glucose) linked together in a crystal structure that strengthens plants and is similar in function to skeletons of animals (Balat *et al.*, 2008). For bioethanol production, cellulose must first be transformed into easily-fermentable monosaccharide (glucoses) before fermentation. As the most common organic compound on Earth, cellulose comprises 38.3%–44.6% of dried banana pseudo-stem (Oliveira *et al.*, 2007) and (Kusmiyati & Sukmaningtyas, 2018).

Lignin: is a highly branched, substituted, mononuclear aromatic polymer in the cell walls of certain biomass, especially woody species, and is often bound to adjacent cellulose fibers to form a lignocellulosic complex. It also provides structural support to the plants, and contributes to the impermeability and resistance against microbial attack (Hendriks & Zeeman, 2009). As Abdullah *et al.*, (2014) and Guerrero *et al.*, (2015) studies, the lignin content in dried pseudo-stem is 8.9% to 37.3%.

Ash: is the mineral content and other inorganic matter in biomass which does not combust during conversion to energy (Pradhan, 2015). Much wood ash contains calcium carbonate as its major component representing 25% to 45%, potash ($\leq 10\%$), phosphate ($\leq 1\%$), iron, magnesium, zinc, copper, and some heavy metals. In banana pseudo-stem, ash account 9.7-24.1% of the dried pseudo-stem see in the table 3.

Table 3: Chemical composition (in %) of banana pseudo-stem as reported in scientific literatures

Reference	Cellulose	Hemicellulose	Lignin	Ash	Extractives
(Guerrero et al., 2015)	38.0 (± 0.5)	8.7 (± 0.1)	8.9 (± 0.2)	17.6 (± 0.0)	24.1(± 3.2)
(Oliveira et al., 2007)	37.3	11.4	13.3	19.0	12.6
(Gabhane et al., 2014)	44.3	22.0	9.6	14.0	10.1
(Abdullah et al., 2014)	44	17.5	37.3	11.0	9.7
(Kusmiyati & Sukmaningtyas, 2018)	44.6%	36.0%	19.39%	-	-

2.7. METHODS FOR COMPOSITION ANALYSIS

In recent years, there are two methods which have been widely used for the determination of lignocellulosic biomass composition; namely Technical Association of the Pulp and Paper Industry (TAPPI) and National Renewable Energy Laboratory (NREL) method (Toribio-

Cuaya et al., 2014). The TAPPI method is useful to characterize the pulp and paper feedstock, while the NREL method is used in the biofuels production.

The main focus of the TAPPI methods are primarily to aid pulp producers to determine the selectivity of chemical pulping, (such as the extent of delignification), maximizing pulp yield and strength as well as determining pulp bleach (Burkhardt *et al.*, 2013). As a result, there is less emphasis on determining a closed material balance or quantifying individual biomass components. For example, rather than using the sulfuric acid hydrolysis method developed by Peter Johan Klason for lignin isolation and quantification, the most commonly employed method used to determine the lignin content and bleach ability of pulps is the indirect permanganate oxidation which does not provide an exact gravimetric measurement of pulp lignin content (McCarthy & Islam, 2000). The detailed tracking of each biomass component through a multi-step process was not a major focus of TAPPI. Thus, TAPPI method for quantifying biomass has tended to be semi-quantitative.

The National Renewable Energy Laboratory (NREL) has developed a comprehensive set of laboratory analytical procedures for characterizing and quantifying biomass (NREL, 2011). The main NREL recommended method for determining an accurate material balance involved modifying the established Klason procedure. It uses a 72% sulfuric acid solution for primary hydrolysis at room temperature followed by dilution with water and a secondary high temperature hydrolysis (Burkhardt *et al.*, 2013). During this two stage analytical procedure, polysaccharides are almost quantitatively hydrolyzed to soluble monosaccharides. This condition leaves most of the lignin behind as an insoluble residue that is washed, filtered and measured gravimetrically. The dissolved monosaccharides are measured using

chromatography techniques and the corresponding carbohydrate polymers are back calculated (NREL, 2008). Because of compositions of lignocellulosic materials such as inorganics (ash), proteins and extractives, an appropriate set of extraction methods are typically needs to remove and quantify these materials (Li *et al.*, 2020).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Description of study area

For this study, three banana varieties: namely giant Cavendishii (*M. acuminata*), dwarf cavendishii (*M. acuminata*), and William-1 (*M. 'Williams hybrid'*) pseudo-stems were selected and used. Those varieties are the most popular and abundantly cultivating varieties in Gamo zone and in Ethiopia as well. All the three banana pseudo-stem varieties were collected under the guidance of Arba Minch ageicultural research center in May, 2021 G.C. The samples were collected from south nation's nationalities and people's regional state, Gamo zone, Arba Minch zuriya wereda, Chano district Ato Abreham Gipo's farm land. Chano is found at distance of 505 km from Addis Ababa at an altitude of 1195 meter and 6° 05' N latitude with 37° 35' E longitude.

Most of the laboratory works of this study were conducted at Hawassa university technology institute school of chemical engineering laboratory and the remaining laboratory works were carried out in biology, chemistry and environmental engineering laboratories in Hawassa University. FT-IR analysis was conducted in Baher Dar university chemistry laboratory and the calorific value of the produced bioethanol was analyzed in Geological survey laboratory Addis Ababa.

3.2. Materials and apparatus

3.2.1. Tools and equipment used

Pole, knife, convective hot air oven, high speed electrical disintegrator (ZAIBA super disintegrator), soxhlet extractor, cellulose thimble (ADVANTEC, No. 84, ID 22 x 80 mm),

round-bottomed flask, volumetric flask (model-BN 3 STAATLICH, Berlin), heating mantel, analytical balance, liquid mixing stirrer, vacuum filter, pH meter (portable pH meter 3310, JENWAY), centrifuge, water bath (GYROMAX 929), solution bottle, autoclave (LaMCS204), beaker, thermometer, magnetic stirrer, scientific calculator, measuring cylinders, bottom round flask, spatula, simple distillation apparatus, UV/Vis spectroscopy (T80- DOUBLE BEAM PC 8 SCANNING AUTO CELL UVD), test tube, ice bath, viscometer (Falling Ball, model 29F), Fourier transfer infrared spectroscopy (Perkin Elmer Spectrum, 65 FTIR), rotary evaporator, bomb calorimeter, shaker incubator (THZ-300C), and furnace were used in this study.

3.2.2. Chemicals and reagent used

All chemicals and reagent used in this study were analytical grade. For this study Distilled water, anhydrous ethanol, sodium hydroxide (NaOH pellet, min. assay 98% BDH Chemicals Ltd pool England), acetic acid (50%v/v), anhydrous methanol, sulphuric acid (H₂SO₄ 96%), hydrogen peroxide (50% H₂O₂), Yeast (*S. cerevisiae*), yeast extract, D-glucose, ammonium sulphate, potassium hypophosphate, magnesium sulphate heptahydrate (MgSO₄.7H₂O), urea, calcium chloride, and potassium dichromate were used.

3.3. Method

3.3.1. Sample preparation

A large amount of banana pseudo-stems from each variety was collected by reducing the size of pseudo-stem using pole. The collected pseudo-stem was chopped out up to average particle size of 5 mm using knife, to facilitate effective drying. Then, the pseudo-stems was sun-dried in an open space for three days (8 hours per day) and it was further dried in oven at 105°C up to constant weight was obtained. The moisture free sample was milled in to powder using high

speed electrical disintegrator. The powdered pseudo-stem sample was used for composition analysis and bioethanol production.

3.3.2 Determination of chemical compositions of banana pseudo-stems

For the present study, determination of the banana pseudo-stems composition was carried out through the modified national renewable energy laboratory (NREL) protocol (Toribio-Cuaya et al., 2014). The amount of each compositions (ash, extractives, hemicellulose, cellulose, and lignin) were calculated through **gravimetric method** (Kusmiyati & Sukmaningtyas, 2018).

3.3.2.1. Ash content

For the determination of ash, 5 g of oven dried banana pseudo-stem samples were incinerated in furnace at 520 °C for 4 hours. The ash of incinerated pseudo-stem was weighted and the ash content was calculated by using equation 1.

$$Ash\ content = \frac{Wt\ of\ ash}{(Wt\ of\ dried\ BS)} * 100 \dots \dots \dots (1)$$

Where,

Wt - Wight

BS – Banana pseudo-stem

3.3.2.2. Extractives

The extractives were determined in two consecutive stages using Soxhlet extractor. During the first stage of extraction, 5 g of dried raw banana pseudo-stem powder was loaded into the cellulose thimble and 190 ml of distilled water was added in to 250 ml round-bottomed flask. The sample loaded thimble was putted into the Soxhlet extraction tube. Then the extraction was carried out at the operation temperature of 100 °C for the reflux time of 5 hours. In this stage, inorganic materials, non-structural sugars, starch and proteins were separated. The extracted extractives with in round-bottomed flask were discarded and the solid with in

thimble was prepared for second stage of extraction. In the second stage, the alcohol extracting extractives remained in solid with in thimble was extracted using anhydrous ethanol (190 mL). It was used as dissolving solvent to separate the compounds such as terpenoids, waxes, fat acids, phenolic substances and chlorophyll; the extraction temperature was 78 °C for the reflux time of 7 hours (Toribio-Cuaya et al., 2014). After extraction, the sample was air dried at room temperature for few minutes. Then after, the solid was further dried in oven at 105°C until constant weight achieved. The extractives amount was the difference in weight between the raw oven dried pseudo-stem sample and extractive-free pseudo-stem sample.

3.3.2.3. Hemicellulose

The extractive free banana pseudo-stem sample was used for determination of hemicellulose. In this study, hemicellulose was determined by alkaline extraction, and the fractions obtained were hemicellulose A and hemicellulose B. The hemicellulose content of each banana pseudo-stems in this study was the sum of the fractions (A and B).

5 g of extractive-free dried banana pseudo-stem was putted into a 1000 ml beaker and 150 ml of 1M sodium hydroxide (NaOH) was added in to the sample with in the beaker. The system was stirred at 130 rpm at room temperature (~26°C) for 24 hours. Then after, the insoluble material was separated through vacuum filtration. Subsequently, the insoluble fraction was washed 3 times with 60 mL of deionized water. To obtain the hemicellulose, the filtrate with pH of 10 was acidified to a pH of 5 by using 50%wt acetic acid. The resultant suspension was stirred for 10 minutes at room temperature and subsequently centrifuged at 3300 rpm for 20 minutes. Then, hemicellulose A was recovered by decantation, the solid was dried to 105°C

for 8 hours in an oven and the weight of the product (dried solid obtained from centrifuge) was measured in analytical balance.

The supernatant (liquid separated via centrifuge) obtained from the first part was concentrated in water bath to a tenth of its original volume (~23 ml). The concentrated of the supernatant was added drop by drop in anhydrous methanol with a ratio of 1:3 in volume to precipitate the hemicellulose B. The mixture was centrifuged at 3300 rpm for 20 minutes. Hemicellulose B was recovered by decanting the methanol supernatant and the recovered solid was dried at 105°C for 8 hours. Then the weight of hemicellulose B was measured. Therefore, the amount of hemicellulose present in the banana pseudo-stem is the sum of fractions A and B.

3.3.2.4. Cellulose

In this study, determination of cellulose content of the pseudo-stem was done by removing lignin (acid soluble and alkaline soluble lignin) and hemicellulose first using the two stages of lignin determination. During the first stage most of the hemicellulose (> 70%) and all the acid soluble lignin are hydrolyzed in an acid media (Toribio-Cuaya et al., 2014). And in the second stage, alkaline lignin and the rest of hemicellulose are extracted thus completely freeing the cellulose.

Stage 1

5 g of dried extractive free banana pseudo-stem was taken in 1000 ml beaker and 150 mL of a 0.275 M of H₂SO₄ solution was added on it. The suspension was stirred at 130 rpm for 24 hours at room temperature. Subsequently the suspension of each variety in the beakers were transferred to solution bottles, then the bottles were sealed with tape and autoclaved at a temperature of 118 °C in a stainless steel steam autoclave for 30 minutes. Once the samples

were autoclaved, vacuum filtration was carried out in order to separate the solid residue. Then, the solid was washed with 60 mL deionized water and the thermochemical treated solid was dried at 40 °C during 8 hours.

Stage 2

The second stage is the delignification process that uses an alkaline media catalyzed with hydrogen peroxide. In order to do so the dried solid of stage one was taken and putted in 1000 ml beaker, 80 mL of 0.375 M of sodium hydroxide (NaOH) solution was added and 1 mL of a solution of 12.8 M H₂O₂ was added. Subsequently the mixture in the beaker was heated at 50°C in hot plate while keeping it in constant stirring (150 rpm) for 3 hours. When the reaction time was over, the solid was recovered by vacuum filtration, and then washed with 60 mL of deionized water as to remove the surplus of NaOH. The remaining solid was the delignified material (it was cellulose + ash), then it was dried at 105 °C for 8 hours. Finally, the mass of delignified dried pseudo-stem sample was measured and recorded.

The amount of cellulose is determined by incinerating the delignified solid at 370°C for 4 hours and it was obtained by subtracting the mass of ash (after incineration) from the mass of delignified dried solid using equation 2.

Cellulose

$$= \text{mass of delignified sample of stage 2} - \text{mass of ash after incineratio} \dots \dots \dots (2)$$

3.3.2.5. Lignin

The lignin content (%wt) was obtained by subtracting the sum of %wt of ash, extractives, hemicellulose, and cellulose from 100% using equation 3.

$$\text{Lignin} = 100\%wt - (\%wt \text{ of (extractives + hemicellulose + cellulose + ash)}) \dots \dots \dots (3)$$

3.3.3. Bioethanol production

The production of bioethanol was done via biochemical conversion method. The method had four major processes; raw material pre-treatment, hydrolysis, fermentation, and distillation. The working parameters in each process were selected based on the best results of previous studies on bioethanol production from banana pseudo-stem. In the present study, the powdered banana pseudo-stem was used as raw material for bioethanol production. Every experimental works in this study were conducted for triplicate.

3.3.3.1. Pretreatment

A large amount of cellulose and hemicellulose content can be converted into bioethanol through a fermentation process where lignin is a barrier for complete conversion. The lignin is resistant to the activity of many microbes and chemical reagents (Hossain *et al.*, 2019). To overcome this circumstance, the dried pseudo-stem was pretreated before scarification (hydrolysis). Pretreatment of the pseudo-stem helps to improve the cellulose accessibility when hydrolysis takes place and to disrupt crystalline structure of cellulose by degrading the lignin. For this study, both physical and chemical pretreatment methods were used.

Physical Pretreatment

The physical pretreatment used was size reduction through milling. It was used because of its ability to disrupt the crystal structure of cellulose and increasing the surface contact. The milling was done by using high speed electrical disintegrator and the powdered sample was sieved through 250 μm mesh. The sample with 250 μm particle size was used for the next processes.

Chemical Pretreatment

As referred in Hossain *et al.*, (2019), the cellulose content of banana pseudo-stem was higher when the pseudo-stem pretreated by 4% NaOH with 12 hours of stirring. As concluded by Hossain *et al.*, (2019), alkaline pre-treatment can remove lignin from banana pseudo-stem effectively. For this reason, alkaline pretreatment using dilute sodium hydroxide (4% NaOH) was used in the present study. The pretreatment was conducted as follows:

4% NaOH solution was prepared, 50g of milled banana pseudo-stem was measured and kept in 1500ml beaker. 800ml 4% NaOH solution was added in to the powder pseudo-stem samples and then, the mixture was steered for 12 hours at room temperature at 120 rpm. Then after the mixture was stirred, it was filtered through vacuum filter. To eliminate the accumulation of salt produced due to the reaction between NaOH and H₂SO₄ which can be happen in hydrolysis; solid residue of filtration reputedly washed by deionized water until the pH become 7. Finally, the solid was dried at 60°C until constant weight obtained.

3.3.3.2. Hydrolysis

According to Hossain *et al.*, (2019) study, enzymatic hydrolysis (using cellulose enzyme) give higher glucose yield than chemical reagent catalyzed hydrolysis. But due to the absence of cellulase enzyme in local chemical market, acid catalyzed hydrolysis was used for this study. Based on the results of Cordeiro *et al.*, (2004) study, higher level of acid concentration and time when using dilute acid hydrolysis, leads to decreasing of bioethanol yield. For the present study, diluted sulfuric acid (H₂SO₄) pre-treatment was applied due to its higher glucose yield ability and method simplicity of de-polymerization of the cellulose in banana pseudo-stem. Along with that, this treatment does not require high-tech and sophisticated equipment (Hossain *et al.*, 2019). In addition, a result of previous studies showed that, dilute acid hydrolysis was more effective than concentrated acid hydrolysis on banana pseudo-stem de-

polymerization (Carlos *et al.*, 2013). Parameters on this experiment were selected based on Hossain *et al.*, (2019) study result, in which maximum de-polymerization achieved.

First, the water bath was switched on and the temperature was adjusted at 92°C to set on it. Subsequently, 1.5% v/v sulphuric acid (H₂SO₄) solution was prepared from 96% H₂SO₄ and 100g of pretreated pseudo-stem sample was measured and putted in solution bottle. 250 ml of 1.5% v/v H₂SO₄ solution was added on the bottle on which the sample found and mixed it well. Then the mixture was putted on water bath when the temperature reaches 92°C and heated for 22 minutes. Finally, the mixture was filtered via vacuum filter and the solid residue was removed.

3.3.3.3. Fermentation

Fermentation is a process by which microorganisms (such as yeast) feed glucose found in the hydrolysate to give alcohol and carbon dioxide as their metabolic byproduct. Microorganisms used in fermentation need a controlled (suitable) environment to feed and grow on it. *S. cerevisiae* is the best fermenting microorganism for bioethanol production from glucose of lignocellulosic biomass. It was used in this experiment by culturing in artificial media for 24 hours. The culture was used in bioethanol production in 1:5 ratios of inoculum to fermentation broth.

Media for yeast (*S.cereviciae*): media for yeast *S.cereviciae* was prepared in 1000ml solution bottle with 500ml working volume. The media was prepared by mixing the following nutrients in their correct proportion (g/L): 10g D-glucose, 3g yeast extract (agar), 5g peptone, 2.5g Urea, 1g MgSO₄.7H₂O, and distilled water (until the mixture become 500ml). Then the pH of the media was adjusted to 5 and sterilized in an autoclave at 121 °C for 15 minutes. 10 ml of yeast

S. cerevisiae (which was obtained from BGI Ethiopia, Hawassa factory) was added after the media was cooled to room temperature. The media with in bottle which contain *S. cerevisiae* was covered with aluminum foil to eliminate the light entering in to the media. Then, it was placed in a shaking incubator for 24 hours, at temperature of 30 °C and 200 rpm (Souza *et al.*, 2012).

Fermentation: *S. cerevisiae* preferred pH 4-6 for fermenting banana waste hydrolysates (fermentation broth) (Matharasi *et al.*, 2018). So that, before the fermentation started, pH of the hydrolysate was adjusted to be 5 using 0.5M NaOH. In the present study, fermentation was carried out in 1000 mL bottle with a working volume of 400 mL (320 mL fermentation broth and 80 mL inoculum), at temperature of 30 °C and initial pH 5. The fermentation was carried out for 72 hours under anaerobic condition (Souza *et al.*, 2012).

3.3.3.4. Distillation

Ethanol was recovered from the fermented broth by the process called distillation. In this study, ethanol was separated by using simple distillation. Simple distillation set up was prepared by assembling condenser, bottom-round flask, and heating mantel together. Then the temperature was set at 78°C and the fermented broth with in bottom-round flask was heated until ethanol completely separated (50-60 minutes) (Hossain *et al.*, 2019). During this time bioethanol was separated from the liquid portion because ethanol has a lower boiling point (78°C) at atmospheric pressure compared to water (100°C).

3.3.4. Characterization of bioethanol produced from the three varieties

Bioethanol was further concentrated via rotary evaporator to use for determination of density, viscosity, flashpoint, calorific value, and for FT-IR analysis. But for determination of alcohol

concentration on produced bioethanol direct output of simple distillation was used. The important physicochemical properties of bioethanol produced from the three varieties were analyzed as follows:

3.3.4.1. Density

Known volume (50 ml) from produced bioethanol was taken in measuring cylinder and then its mass was measured. The density was calculated by dividing bioethanol mass by its volume (equation 4).

$$Density = \frac{mass}{volume} \dots \dots \dots (4)$$

3.3.4.2. Viscosity

The viscosity of produced bioethanol was measured using (Falling Ball, model 29F) viscometer. Before starting the measurement, the viscometer was adjusted at an angle of 10°. Then after, bioethanol sample was filled inside the glass tube of viscometer and the viscometer ball was fall down from the top of glass tube. After that, the viscometer was turned around at an adjusted angle and then stop-watch was started counting time. The time at which the ball reaches the bottom of the glass tube was recorded from stop-watch. Finally, dynamic viscosity and kinematic viscosity was calculated using equation (5) and (6) respectively.

$$\eta = k(\rho_1 - \rho_2) * t \dots \dots \dots (5)$$

Where,

η - Dynamic viscosity (mPa.s)

k- Ball constant = 0.09 mPa.s

ρ_1 - Density of the ball (2.2g/cm³)

ρ_2 - Density of bioethanol(g/cm³)

t- Time (second)

μ - Kinematic viscosity (cSt or mm²/s)

$$\mu = \frac{\eta}{\rho} \dots \dots \dots (6)$$

3.3.4.3. Flash Point

Flash point of the produced bioethanol was determined using open cup method. 10 ml of concentrated bioethanol was taken in pan and kept in the ice bath until its temperature reached 10 °C. A heating mantel was switched on and the temperature was adjusted at 50 °C. The pan which contains bioethanol was placed on to heating mantel, the electrode of thermometer was inserted in to it and subsequently bioethanol was heated. When heating the bioethanol, ignition temperature of the fuel was tested at each temperature increase, by flashing fire through lighter. The test was continued until the fuel ignites. Finally, the temperature at which the fuel ignited was recorded.

3.3.4.4. Calorific value

The calorific value analysis of bioethanol was carried out by using bomb calorimeter, in Geological Survey laboratory, Addis Ababa. The process of a calorimeter involves measuring the heat of a fuel sample when fuel burned under stable temperature conditions to evaluate the heating energy of the fuel sample. The calorimeter used for this study was measure and recorded the date automatically and finally it gave the calculated results. The analysis was conducted for the bioethanol produced from each variety as follows:

1g of bioethanol was measured and placed in a small stainless steel crucible. The crucible containing the fuel was then placed inside the bomb. The bomb was sealed air tight by the lid. The sealed bomb was then placed in a large well-insulated copper calorimeter in which water

was poured to absorb heat released when the fuel combusted. Then the water was kept in constant agitation by mechanical stirrer to maintain constant temperature distribution within calorimeter. Then, the bomb was filled with oxygen and combustion of the fuel was initiated by passing electric current through the ignition coil. When the sample burnt in the bomb, heat was liberated and was absorbed by the surrounding water and copper calorimeter. And then the maximum temperature at which the gradual increment becomes constant was noted as final temperature. Finally, the calorimeter calculates and gave the calorific value automatically.

3.3.4.5. Bioethanol concentration

The concentration of produced bioethanol was determined through T80 UV/Vis spectroscopy. UV/Vis spectroscopy does not give the concentration directly; concentration was obtained from calibration curve which was plotted using absorbance and concentration of standard ethanol. Before measuring absorbance via UV/Vis spectrometer, it was needed to do the following tasks:

Potassium dichromate ($K_2Cr_2O_7$) solution preparation- it was prepared in 1000 ml volumetric flask from 325ml of 96% H_2SO_4 , 33.8g of potassium dichromate ($K_2Cr_2O_7$) and distilled water (until total solution reached 1000 ml).

Standard ethanol solution preparation- 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 %(v/v) standard ethanol solution was prepared from 99.99%(v/v) ethanol, 1ml from each standard was took within 8 test tubes and 9ml of distilled water was added into each test tubes. Then after, 1ml from each test tube was taken in to another 8 test tubes and 2 ml of potassium dichromate solution was added..

Heating the samples- test tubes which contain 1ml standard ethanol and 2ml potassium dichromate solution was heated in water bath at 60°C for 20 minutes and then rapidly cools down.

Measuring absorbance- all the parameters in spectrometer required for measurement was adjusted and then the absorbance of heated standard ethanol mixture was recorded for 3 replication at 600nm (Singh, 2015). Then after, the calibration curve was plotted in excel sheet and its slop and intercept was calculated. Finally, the concentration of produced bioethanol was determined from prepared calibration curve of standard ethanol using equations (7) and (8).

$$Y = mx + b \dots \dots \dots (7)$$

Where; y = absorbance; x = ethanol concentration

m = the slop and b = y- intercept

conc. of unknown sample(trsc)

$$= \frac{\text{absorbance of unknown sample}(y) - y \text{ intercept}(b)}{\text{slop}(m)} \dots \dots \dots (8)$$

3.3.5. FT-IR analysis

Bioethanol produced (from the three varieties) through fermentation was analyzed by using prinks Elmer Spectrum 65 Fourier Transform Infrared (FT-IR) spectroscopy. The analysis was done to identify the functional groups found in the produced bioethanol. In FTIR, the transmittance (T) was recorded in the form of %T and the wavenumber region for the analysis

was recorded at 4000cm⁻¹ to 400cm⁻¹ in IR spectrum. Then, the percentage transmittance (%T) versus wavenumber (cm⁻¹) was recorded and the spectrum was plotted using origin lab software and the functional group present in the sample was identified by interpreting the spectrum. The analysis was conducted in Bahir Dar University, Chemistry department.

3.3.6. Bioethanol yield estimation

The bioethanol yield was determined based on the produced bioethanol content, which was obtained using calibration curve constructed from standard ethanol solution using potassium dichromate method. Bioethanol yield from the three varieties was determined by using equation 9 and compared each other:

$$\text{Bioethanol yield (\%)} = \left(\frac{Vb}{Vfb} \right) \times 100 \dots \dots \dots (9)$$

Where; *Vb*= volume of produced bioethanol

Vfb= volume of fermentation broth used in fermentation

3.4. Statistical analysis

The experiment was consisted on three varieties of banana pseudo-stem with three numbers of replications. The data analysis was done by using Statistical analysis of variance (one way ANOVA) through statistical analysis software (SAS) to test the variety effects on dependent variables. Mean comparison was done using Least Significant Difference (LSD) at 5% significance level..

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Chemical compositions of banana pseudo-stems

4.1.1. Ash content

Laboratory result of this study on ash content implied, giant cavendishii and Dwarf cavendishii pseudo-stems have different ash content than William-1 as shown in figure 1 and table 7. The ash fraction obtained was $7.99\pm 0.09\%$ (giant cavendishii), $8.17\pm 0.05\%$ (dwarf cavendishii) and $10.12\pm 0.01\%$ (William-1). All these values are lower than the studies reported in table 3.

4.1.2. Extractives

Determination of extractive within the pseudo-stem was the first step of composition determination. Extractives in the pseudo-stem, was determined through soxhlet apparatus using water and ethanol as extracting solvent. Extractives content of giant cavendishii, dwarf cavendishii and William-1 obtained from laboratory work were $27.69\pm 0.09\%$, $27.25\pm 0.01\%$, and $31.15\pm 0.01\%$, respectively. As observed in the result, William-1 comprised of the highest amount of extractives than the remaining two varieties. However, there is no significant difference between giant cavendishii and dwarf cavendishii. In general, the extractive content in willam-1 was significantly different ($P < 0.0001$) than the remaining two varieties (appendix table 2). The results of this study were consistent with the results reported on previously done researches by Oliveira *et al.*, (2007) and Guerrero *et al.*, (2015). Who reported that the extractive content of dried banana pseudo-stems was in the range from 12.60% to 24.10%.

4.1.3. Hemicellulose

The percentages of hemicellulose in the giant cavendishii, dwarf cavendishii and William-1 were $19.37 \pm 0.02\%$, $23.85 \pm 0.09\%$ and $19.32 \pm 0.01\%$, respectively. As SAS output, there is no significant difference in hemicellulose content of giant cavendishii and Willam-1. But hemicellulose in dwarf cavendishii was slightly higher than the remaining two varieties. i.e there is significant difference ($Pr. < 0.0001$) of hemicellulose between the varieties. According to the former researchers finding provided in table 3, the hemicellulose composition was ranging from 8.7% to 36.0% Guerrero *et al.*, (2015) and Kusmiyati & Sukmaningtyas, (2018). Thus, hemicellulose content of the three varieties in the current research was within the range, especially hemicellulose in dwarf cavendishii was the same as compared with the report of Gabhane *et al.*, (2014).

4.1.4. Cellulose

Cellulose content obtained in the giant cavendishii, dwarf cavendishii and William-1 was $36.14 \pm 0.09\%$, $31.71 \pm 0.03\%$ and $30.11 \pm 0.01\%$, respectively. The values of dwarf cavendishii and William-1 are slightly lower than the result of composition studied by Oliveira *et al.*, (2007) in table 3. But cellulose composition of giant cavendishii was almost similar with Oliveira *et al.*, (2007). Oliveira *et al.* reported that the banana pseudo-stems had an approximated percentage of 37.30% of cellulose, which is very similar to the one obtained in this study. In some researches such as Gabhane *et al.*, (2014), larger amounts of cellulose have been reported, which can be either due to the consequence of mixing different pseudo-stem varieties in one or the cultivation conditions (like soil type, temperature, etc...) of the banana plan. In the present study, the fractions (compositions) were analyzed depending on pseudo-stems variety only. The SAS output implied that the three pseudo-stems have significantly

different cellulose composition (appendix table 4), Giant cavendishii bear the largest cellulose content.

4.1.5. Lignin

Determination of lignin content in the pseudo-stems was carried out after determination of other contents, through calculation using equation 3. The lignin composition in giant cavendishii, dwarf cavendishii and William-1 were $8.81 \pm 0.09\%$, $9.02 \pm 0.03\%$ and $9.30 \pm 0.04\%$ respectively. The result shows that the three varieties have different lignin composition (appendix table 5) but the results were consistent with Gabhane *et al.*, (2014) and Guerrero *et al.*, (2015) studies.

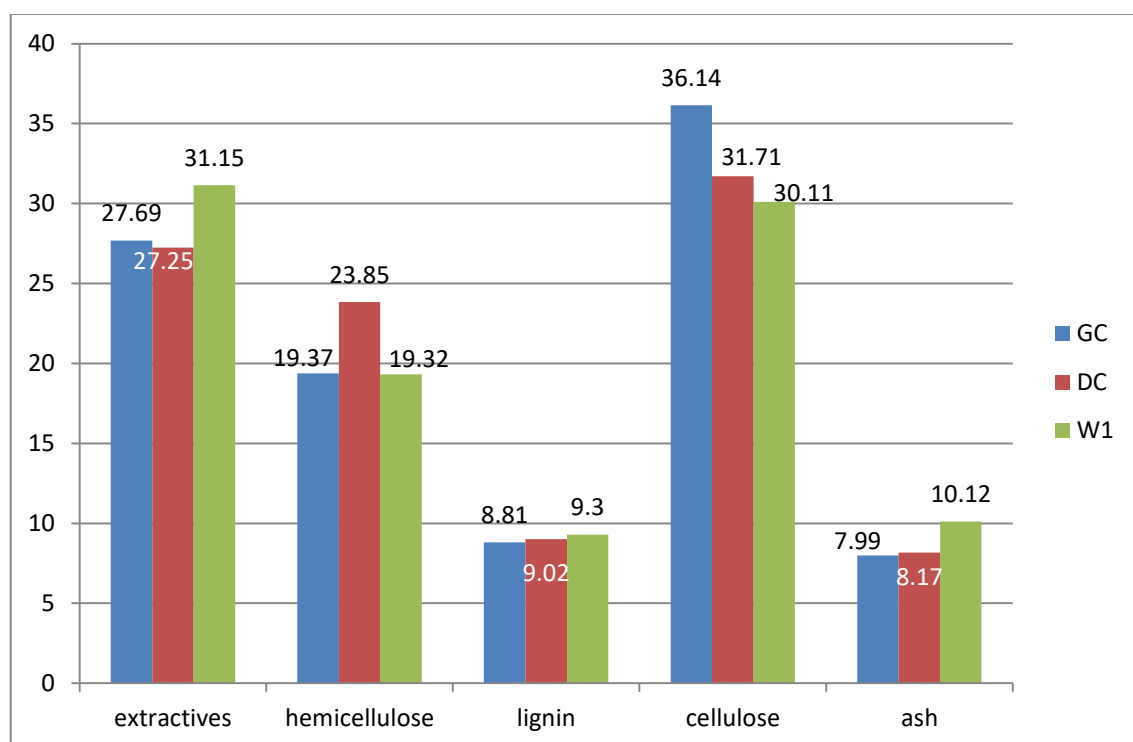


Figure 1: Chemical composition of the three varieties banana pseudo-stem (GC-giant cavendishii, DC- dwarf cavendishii, W1- William-1)

In general, from the laboratory result of composition analysis shown in figure 1, giant cavendishii banana pseudo-stem has lower lignin and ash content, and higher cellulose. In

dwarf cavendishii, the lignin and ash content was slightly higher and cellulose was lower than giant cavendishii while William-1 has higher lignin and ash, and lower cellulose content. Obviously, the presence of higher lignin and ash in the pseudo-stem, negatively affect bioethanol yield through being inhibitor for microbial activity. In opposite to this, the higher cellulose content promote highest bioethanol yield because of higher glucose obtained through hydrolysis.

4.2. Produced bioethanol characterization results

4.2.1. Density

The mass of 50 ml bioethanol of giant cavendishii, dwarf cavendishii and William-1 obtained was 41.95g, 41.60g, and 41.85g. Using equation (4), density was 0.839 g/cm³, 0.832 g/cm³, and 0.837 g/cm³, respectively. Bioethanol density of all the three varieties was different from the result of Toor *et al.*, (2019) study. This was due to the difference in concentration, the sample in Toor *et al.*, (2019) had 99.5% but bioethanol of this study had 80.1% concentration.

4.2.2. Viscosity

The result obtained from viscometer was falling time (in second), at which the failed ball reach the bottom of glass tube. Falling time of bioethanol from giant cavendishii, dwarf cavendishii and William-1 were 9.39sec, 9.38sec, and 9.38sec, respectively. The calculated result of kinematic viscosity for giant cavendishii, dwarf cavendishii, and William-1 were 0.965 cSt, 0.961 cSt, and 0.962 cSt, respectively. The results were similar; this was because the produced bioethanol was concentrated through rotary evaporator to have the same concentration. Those calculated results were lower than the previous study of Toor *et al.*, (2019). This difference was made due to the concentration difference i.e. bioethanol in Toor *et al.*, (2019) study had 99.5% but the sample of this study had 80.1% alcohol concentration.

4.2.3. Flash point

Bioethanol produced from the three varieties was concentrated through rotary evaporator. Concentrating was important to minimize the temperature at which the fuel starts to ignite. For fuels with higher moisture content, flash point must be larger. The flash point was determined according to the method provided in previous chapter and the result of the three varieties becomes 14.5°C. The result obtained was slightly higher than Toor *et al.*, (2019) study, this was due to the presence of considerable amount of water on the sample.

4.2.4. Calorific value

The analysis was completed within 4 minutes and the calculated results of calorimeter for giant cavendishii, dwarf cavendishii, and William-1 were 21.60MJ/kg, 21.59MJ/kg, and 21.60MJ/kg respectively. The result shows that the calorific value of bioethanol of the three varieties was almost similar. As compared with other studies, the results obtained here were lower than the calorific value of pure ethanol found in Toor *et al.*, (2019) study shown in table 1. But all the results of this study were consistent with Okoro *et al.*, (2012) study.

4.2.5. Concentration of produced bioethanol

In present study, bioethanol was produced from the three varieties of banana pseudo-stem through separate saccharification and fermentation and the concentration was determined from calibration curve. The calibration curve was plotted using the absorbance of prepared standard ethanol solutions (figure 2). From this curve, a linear equation was developed, through regression the slope and y-intercept become 2.1707 and 0.0415, respectively. Figure 2 shows that the calibration curve was plotted by standard ethanol concentration versus absorbance measured at 600 nm. The concentration of bioethanol produced was then calculated by using equation 8. Bioethanol concentration after subtracting ethanol produced by inoculum (7.1%),

were 20.2% v/v, 16.6% v/v and 12.8% v/v for giant cavendishii, dwarf cavendishii and William-1, respectively.

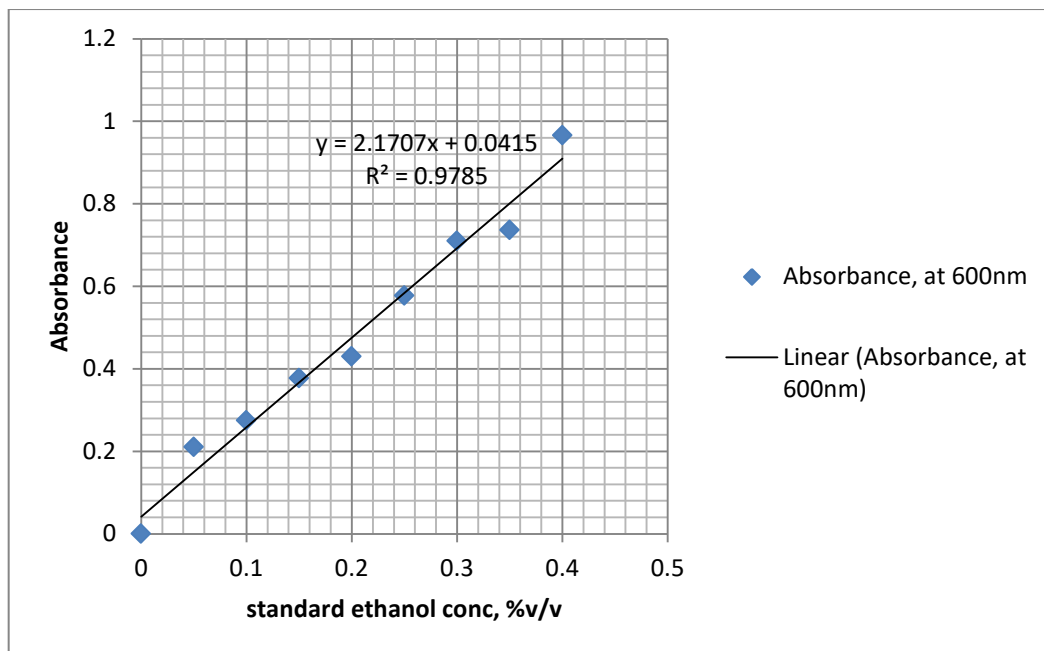


Figure 2: The calibration curve of standard ethanol

4.3. FT-IR identification of -OH functional group

The association of -OH, C-H, and C-O stretching vibrations signify characteristic of IR transmittance of alcohols. In this study, table of IR frequency range was used to determine the identity of substances found in the produced sample. The IR spectrums of bioethanol produced from the three pseudo-stems were presented in the figure 3 bellow. As shown in the figure 3, the broad peak in the region of $4000-3000\text{cm}^{-1}$ specified the O-H stretch of alcohol. But the presence of O-H stretch cannot give enough information to say the analyzed sample was ethanol (alcohol), because in addition to alcohol carboxylic acids and phenols have O-H functional group on their structure. Reading on the region $1100-1000\text{cm}^{-1}$ proves the C-O stretch, it showed that the analyzed sample was not carboxylic acid, it is may be alcohol or

phenol (table 4). The peak at about $3000\text{-}2800\text{cm}^{-1}$ exactly differentiate the analyzed sample was alcohol; the spectrum signified the presence of $-\text{CH}_2-\text{CH}_3$ groups in the analyzed samples of this study. This condition tells that the product obtained from the three banana pseudo-stems was absolutely ethanol due to the verification of these regions (O-H, C-O and C-H) (Figure 3).

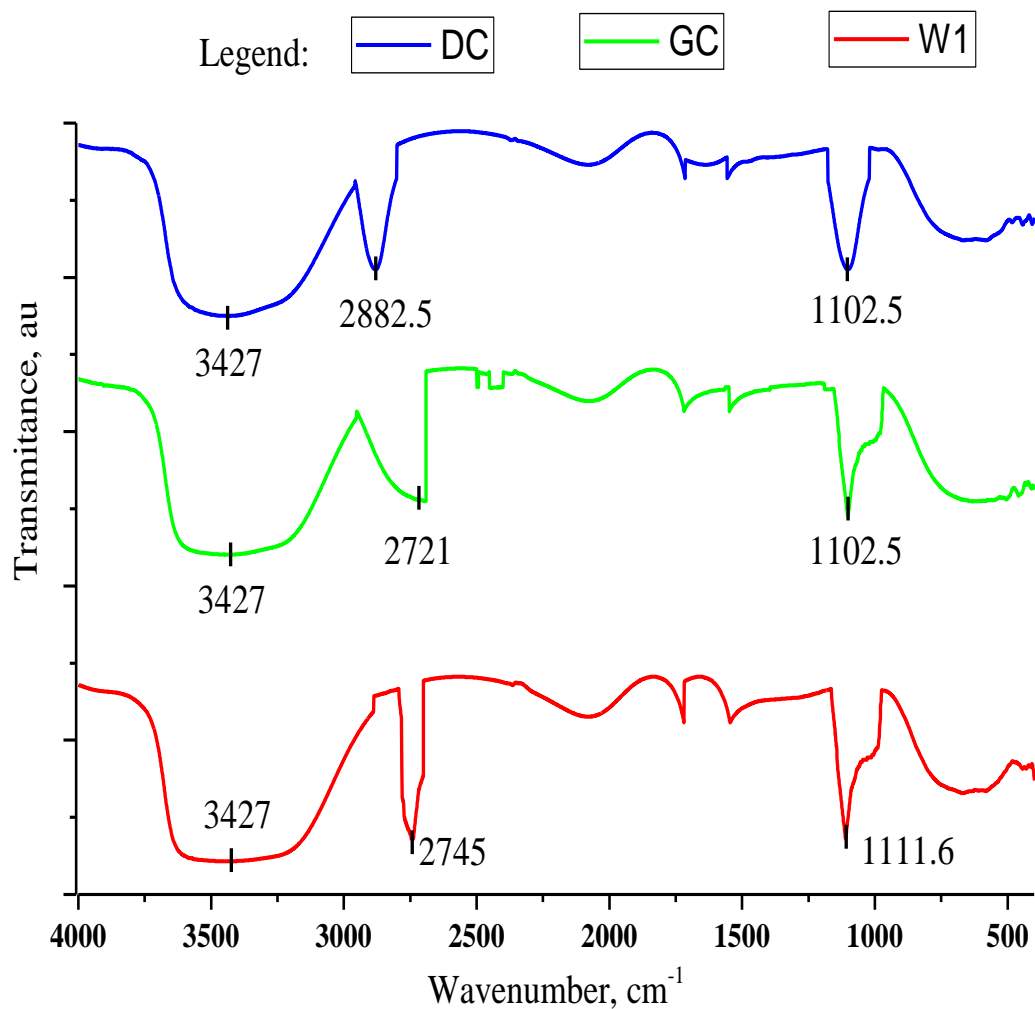


Figure 3: FTIR spectrum of produced bioethanol

Table 4: IR spectroscopy correlation comparison of alcohols, carboxylic acids, and phenols

Functional group	Acid	Alcohol	Phenol
O-H	3600cm ⁻¹	3600cm ⁻¹	3400cm ⁻¹
C-O	1200cm ⁻¹	1100cm ⁻¹	1060cm ⁻¹
C=O	1730cm ⁻¹	-	-
C-H	-	2800cm ⁻¹	-

Table 5: Summary of tested physicochemical characteristics of produced bioethanol

Variables	Giant cavendishii	Dwarf cavendishii	William-1
Density (g/cm ³)	0.839	0.832	0.837
Kinematic viscosity (cSt)	0.965	0.961	0.963
Calorific value(MJ/kg)	21.60	21.59	21.60
Flash point (°C)	14.5	14.5	14.5
Alcohol concentration (%)	20.2	16.6	12.8
Alcohol identification in FTIR	Identified	Identified	Identified

4.4. Bioethanol yield

Percent yield of bioethanol from each pseudo-stem varieties was calculated using equation 9. For the % yield determination, Vb was 20.2ml for giant cavendishii, 16.6ml for dwarf cavendishii and 12.8ml for William-1 and Vfb was 320ml. After calculation, %yield of giant cavendishii, dwarf cavendishii and William-1 was 6.31, 5.2 and 4.0, respectively (table 6). The mean comparison on SAS signified that there is significant difference on bioethanol yield between the three varieties (Pr.<0.0001). Result of this study implies giant cavendishii bear the largest value of % yield. As compared with Guerrero *et al.*, (2015) study, results of the present study was much lower. This is because in Guerrero *et al.*, (2015) the hydrolysis used was enzymatic (cellulase and hemicellulase enzymes) which rise the amount of fermentable sugar. Beside hydrolysis, the fermentation was also carried out by using microorganisms which ferment 5- carbon and 6- carbon sugars obtained from hemicellulose in addition to *S. cerevicae*. But the present study focus on utilizing only cellulose, hydrolyzed by dilute acid method, and *S. cerevicae* was the only microorganism used for fermentation.

Table 6: Pure bioethanol obtained from distillation process and % yield

Variety	Item	Unit
Giant cavendishii	Distilled pure bioethanol (mL)	20.2
	Total fermented solution (mL)	320
	Bioethanol yield (mL bioethanol/mL fermented solution)*100%	6.31%
Dwarf cavendishii	Distilled pure bioethanol (mL)	16.6
	Total fermented solution (mL)	320
	Bioethanol yield (mL bioethanol/mL fermented solution)*100%	5.20%
William-1	Distilled pure bioethanol (mL)	12.8
	Total fermented solution (mL)	320
	Bioethanol yield (mL bioethanol/mL fermented solution)*100%	4.0%

Table 7: Bioethanol yield and chemical composition (mean value \pm SD) of the three varieties of banana pseudo-stems (from 100%)

Varieties	BEY	EXT	HEM	LIG	CEL	Ash
GC	6.31 ^A \pm 0.11	27.69 ^B \pm 0.09	19.37 ^B \pm 0.02	8.81 ^C \pm 0.09	36.14 ^A \pm 0.09	7.99 ^B \pm 0.09
DC	5.20 ^B \pm 0.10	27.25 ^B \pm 0.01	23.83 ^A \pm 0.09	9.02 ^B \pm 0.03	31.71 ^B \pm 0.03	8.17 ^B \pm 0.05
William-1	4.00 ^C \pm 0.05	31.15 ^A \pm 0.01	19.32 ^B \pm 0.01	9.30 ^A \pm 0.04	30.11 ^C \pm 0.06	10.12 ^A \pm 0.01
CV	1.75	0.183	0.252	0.582	0.161	0.928
Pr.>F	0.0001	0.0001	0.0001	0.0001	0.0001	0.0204
ANOVA decision	Significan t	Significant	Significant	Significant	Significant	Significant

Where, GC- Giant cavendishii, DC- Dwarf cavendishii, BEY-bioethanol yield, EXT-extractive, HEM- hemicellulose, CEL- cellulose, LIG- lignin, CV-Coefficient of Variation, ANOVA-analysis of variance and Values are expressed as mean and means in different letters imply the means are significantly different while the same letters imply the mean are the same according to list significant difference (LSD) test

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1. CONCLUSION

Banana pseudo-stem has been evaluated for bioethanol production in many countries in the world. But all the studies done to evaluate the pseudo-stems were without considering banana pseudo-stems varieties. This study was strived to characterize and evaluate bioethanol potential on the pseudo-stem of most commonly cultivating banana varieties in Ethiopia, based on a laboratory analysis. Since bioethanol potential of pseudo-stem is basically depends on its cellulose content and bioethanol yield, this study emphasized on pseudo-stem composition analysis and bioethanol production from the pseudo-stem to determine bioethanol yield.

In this study the composition of pseudo-stems were analyzed using NREL protocol and calculated through gravimetric method. And also, bioethanol yield was determined by producing bioethanol from pseudo-stem of each variety. The result obtained was analyzed and compared through SAS software. As experimental outcomes, the average cellulose content of giant cavendishii, dwarf cavendishii, and William-1 were $36.14^A \pm 0.09$, $31.71^B \pm 0.03$, and $30.11^C \pm 0.06$, respectively. Mean separation in SAS software signified that the cellulose composition of the three varieties were different; giant cavendishii has larger cellulose content. Besides the highest cellulose content, the mean value of bioethanol yield in giant cavendishii was larger ($6.31\%^A \pm 0.11$) followed by dwarf cavendishii ($5.20\%^B \pm 0.10$) and William-1 ($4.00\%^C \pm 0.05$). This is evident that giant cavendishii has the highest bioethanol potential. Therefore, giant cavendishii banana pseudo-stem is the preferred feedstock for bioethanol production than other varieties which were covered in this study.

5.2. RECOMMENDATION

Based on the result of the present study, the following recommendations are forwarded:

- ❖ Bioethanol production in this study was relied only on utilizing glucose (building block of cellulose), that's why *S. cerevisiae* was used for fermentation. This circumstance was limiting the maximum amount of bioethanol obtained from the pseudo-stems. So that, researches on bioethanol fermentation through co-culture of different microorganisms (which ferment 5 carbon and 6 carbon sugar of hemicellulose) in addition to glucose of cellulose must be help to boost the overall bioethanol yield on pseudo-stems of each banana varieties.
- ❖ Pseudo-stem samples used in this study was collected from the same farm land (means they cultivated in the same soil type, same climatic condition, etc). Additional studies which consider those factors, give extra evidence to select the preferable pseudo-stem varieties.
- ❖ Giant cavendishii contains relatively high cellulose with low lignin content, it should be preferable feedstock for bioethanol production.

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APPENDIX

Appendix table 1: ANOVA table for bioethanol yield (one way ANOVA)

Source of variation	DF	SS	MS	F-Value	Pr.>F	CV
Variety	2	8.01	4.00	488.30	< .0001	1.75
Error	6	0.05	0.01	-	-	-
Total	8	8.06	-	-	-	-

Appendix table 2: ANOVA table for extractive (one way ANOVA)

Source of variation	DF	SS	MS	F-Value	Pr.>F	CV
Variety	2	27.36	13.69	4947.33	< .0001	0.18
Error	6	0.02	0.00	-	-	-
Total	8	27.38	-	-	-	-

Appendix table 3: ANOVA table for hemicellulose (one way ANOVA)

Source of variation	DF	SS	MS	F-Value	Pr.>F	CV
Variety	2	40.23	20.12	7271.24	< .0001	0.25
Error	6	0.02	0.00	-	-	-
Total	8	40.25	-	-	-	-

Appendix table 4: ANOVA table for cellulose (one way ANOVA)

Source of variation	DF	SS	MS	F-Value	Pr.>F	CV
Variety	2	58.55	29.27	10,580.6	<0.0001	0.161
Error	6	0.02	0.001	-	-	-
Total	8	58.57	-	-	-	-

Appendix table 5: ANOVA table for lignin (one way ANOVA)

Source of variation	DF	SS	MS	F-Value	Pr.>F	CV
Variety	2	0.36	0.18	65.53	<0.0001	0.582
Error	6	0.02	0.00	-	-	-
Total	8	0.38	-	-	-	-

Appendix table 6: ANOVA table for Ash (one way ANOVA)

Source of variation	DF	SS	MS	F-Value	Pr.>F	CV
Variety	2	6.18	3.09	7.98	0.02	6.93
Error	6	2.32	0.39	-	-	-
Total	8	8.50	-	-	-	-

Appendix table 7: summarized laboratory results of the three banana variety pseudo-stems with three replication

Variety		BEY (%)	EXT (%)	HEM (%)	CEL (%)	LIG (%)	ASH (%)
GC	R1	6.20	27.60	19.46	36.23	8.72	8.08
	R2	6.31	27.69	19.37	36.14	8.81	7.99
	R3	6.42	27.78	19.28	36.05	8.90	9.90
DC	R1	5.10	27.24	23.84	31.72	9.01	8.16
	R2	5.20	27.25	23.83	31.71	9.02	8.17
	R3	5.30	27.26	23.82	31.70	9.03	8.18
W1	R1	3.95	31.14	19.31	30.10	9.39	10.11
	R2	4.00	31.15	19.32	30.11	9.30	10.12
	R3	4.05	31.16	19.33	30.12	9.31	10.13

Where, BEY-bioethanol yield, EXT-extractive, HEM-hemicellulose, CEL-cellulose, LIG-lignin, ASH-ash content



(a)



(b)



(c)

Appendix figure1: (a)- banana pseudo-stem collection, (b)-the first day of sun drying banana pseudo-stem, and (c)-identifying banana varieties on farm



(a)



(b)

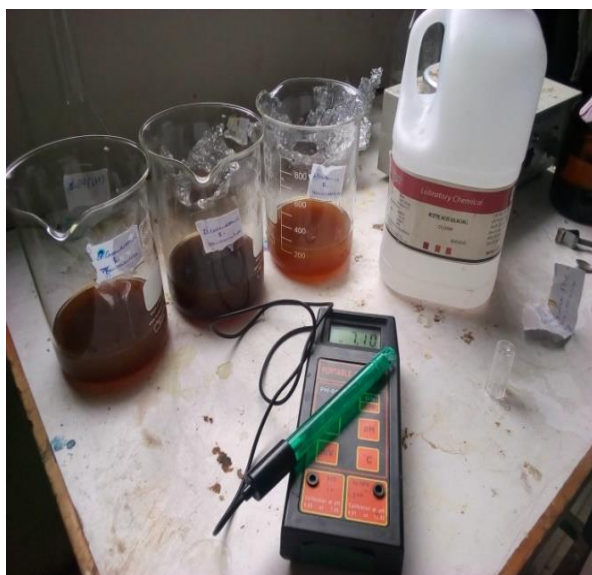


(c)

Appendix figure 2: (a)-three day dried pseudo-stem, (b)-Soxhlet extractor, and (c)-Extracting extractives



(a)



(b)

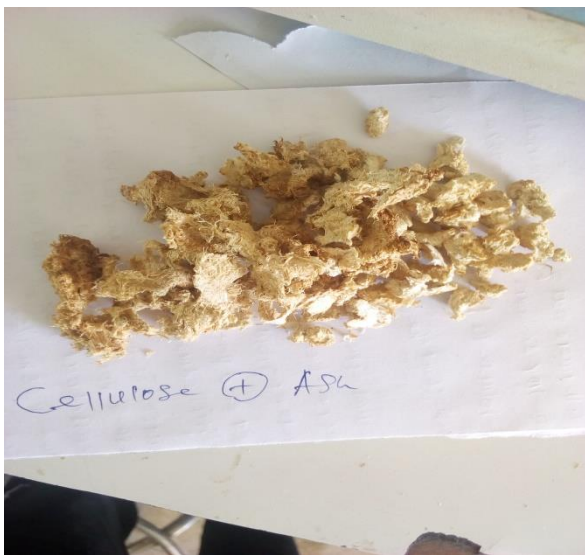


(c)

Appendix figure 3: (a)-extracted extractives, (b)-pH adjustment during hemicellulose determination, and (c)-mixture of hemicellulose B and methanol



(a)



(b)



(c)

Appendix figure 4: (a)-vacuum filtration, (b)-cellulose + ash, and (c)- pretreated pseudo-stem sample



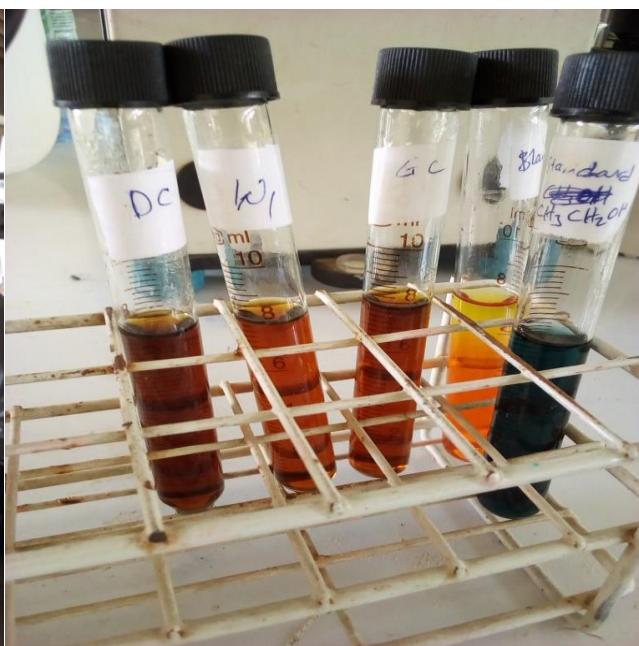
(a)



(b)



(c)



(d)

Appendix figure 5: (a)-cellulose separated through vacuum filtration, (b)-separating alkaline soluble lignin to get cellulose, (c)-mixing through liquid mixer, and (d)-produced bioethanol and potassium dichromate mixture for UV/Vis concentration determination

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